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PHYTOPLANKTON STUDIES IN THE LITTLE MANATEE RIVER  
SPECIES COMPOSITION, BIOMASS, AND NUTRIENT  
EFFECTS ON PRIMARY PRODUCTION

Gabriel A. Vargo  
Department of Marine Science  
University of South Florida

Funds for this project were provided by the Florida Department of Environmental Regulation, Office of Coastal Management using funds made available through the National Oceanic and Atmospheric Administration under the Coastal Zone Management Act of 1972, as ammended. Local administration of this work was conducted through contracts with the Southwest Florida Water Management District. Project Manager: Michael S. Flannery.

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## EXECUTIVE SUMMARY

The objectives of the phytoplankton component of the Little Manatee River project were to investigate phytoplankton species composition and production in the estuary and to assess any limiting nutrient conditions and the potential for eutrophication. Field collections for the phytoplankton study were done simultaneously with water quality sampling between January 1988 and January 1989. At bi-weekly intervals, nutrients, salinity, light penetration, chlorophyll a and phytoplankton counts were measured in the 0 ppt, 6 ppt, 12 ppt, and 18 ppt salinity zones plus Tampa Bay and Ruskin Inlet. Also, at all stations except 6 ppt and Ruskin Inlet, primary production was measured on incubated water samples and combinations of nitrogen and phosphorus were added to assess nutrient limitation.

River flows during the study year were characterized by prolonged low flows in the spring and high flows in the summer, most notably a major flood during September which flushed the entire river with fresh water and significantly reduced salinity in Tampa Bay. Light attenuation, expressed as extinction coefficients, was greatest at the low salinity stations and lowest near the mouth of the river. Extinction coefficients were positively related to streamflow, being lowest during the spring dry period when color concentrations in the river were reduced.

Chlorophyll a concentrations in the river showed a decreasing trend with salinity with the greatest seasonal variation occurring at the low salinity stations (0 ppt and 6 ppt). Concentrations were generally lower and less variable in the 12 ppt and 18 ppt salinity zones. High river flows during August and September reduced chlorophyll concentrations to their lowest values throughout the estuary, but maximum concentrations were recorded at most stations shortly thereafter during a period of decreased river flow which allowed restabilization of the water column.

The phytoplankton community in the Little Manatee River and adjacent area of Tampa Bay consisted primarily of a seasonally varying mixture of diatoms, dinoflagellates, microflagellates and chlorophytes with a sporadic occurrence of

blue-green algae. With the exception of a few dates, diatoms or microflagellates were the dominant groups at all stations. Many algal species were found to occur regularly throughout the estuary, suggesting that upriver transport of bay water with accompanying cells occurs regularly. However, pronounced spatial differences in species composition were observed in the estuary and periodic blooms occurred at different times in the various salinity zones. Diatoms were most consistently abundant at the Tampa Bay station where they averaged 44.8% of total cell counts. Microflagellates were the co-dominant group in the bay except during a late summer-fall bloom of the blue-green alga, Schizothrix, which occurred in the bay and river after the September flood. Microflagellates were generally the dominant group at all river stations, particularly the intermediate salinity zones (12 and 18 ppt) and the low salinity zones during the summer. Estimates of total cell volume, however, showed that this group of small, unidentified species usually comprised less total volume than the diatoms. Of all the river stations, total phytoplankton counts were highest for the 0 ppt salinity station. Diatom blooms were more frequent at this station compared to intermediate salinity zones. Chlorophytes were most abundant at this station with a maximum abundance of 7% of total cells in late September. High phytoplankton counts and chlorophyll concentrations at the 0 ppt station were most likely due to primary production within the upper estuary since chlorophyll concentrations at the most downstream freshwater site were consistently low. Chlorophyll and phytoplankton counts were reduced at the 0 ppt station during the summer, due possibly to flushing of the upper estuary by medium to high river flows. Of all the study sites, total phytoplankton counts were highest at the Ruskin Inlet station, a eutrophic, channelized inlet to the river 2.5 miles above its mouth. At this station there was an almost continuous presence of euglenoid flagellate species which are phagotrophic and indicative of high organic particle loads.

Primary production rates in the river generally followed trends observed for chlorophyll concentrations and cell counts, with highest values occurring at the 0 ppt station and progressively decreasing values found in the 12 ppt and 18 ppt salinity zones. In short-term (3 hour) experiments, enrichment of samples from Tampa Bay and three river stations with combinations of nitrate and phosphate did not show a consistent enhancement of photosynthesis. Additions of ammonium to dark incubated samples gave results indicating that waters from

Tampa Bay and the river could be considered nutrient sufficient or borderline nitrogen-limited with regard to short-term photosynthesis. Ambient water column concentrations of ammonium, nitrate and phosphate indicated that phosphorus was always present in excess, while ammonium and nitrate concentrations varied considerably and at times were at the limit of detection.

The results indicate that short-term phytoplankton production in the estuary is not usually nutrient limited, but long-term nutrient limitation of growth or biomass may occur. Ongoing second year experiments conducted on waters from the 12 ppt zone have shown that nitrogen additions alone can result in dramatic long-term increases in chlorophyll levels. Although short-term photosynthesis may not be limited with regard to ambient nutrient levels, increased nitrogen loading should result in increased phytoplankton levels in the estuary and the potential for eutrophication if such loading occurs is high.



## INTRODUCTION

The Little Manatee River project was designed as a multi-disciplinary, multi-agency study of a moderately impacted watershed in the Tampa Bay estuarine system. The first year of this study was designed to provide basic information about the physical, chemical and biological components of the river that could be used for future management decisions.

The objectives of the phytoplankton component of this study are to provide information on the seasonal and spatial variation in species composition and to conduct nutrient addition experiments to assess potential nutrient limitation of this community and the potential for eutrophication in the Little Manatee River.

## METHODS

Water samples for phytoplankton abundance and species composition were collected twice monthly at 5 locations in the Little Manatee River and at a station in Tampa Bay, approximately 2.3 miles northeast of the mouth of the river (Fig. 1). Additional water samples for determining the effect of nitrogen and phosphorus additions on carbon-14 uptake by these same populations were taken at 3 of the river stations and at the Tampa Bay station. Tampa Bay and Ruskin Inlet, in the Little Manatee River, were the only fixed locations. Ruskin Inlet is a channelized inlet off the Little Manatee River which is surrounded by urban development (Fig. 1). The remaining station locations were determined by salinity so their geographic location varied throughout the year (see Table 1). Phytoplankton abundance and species composition were determined at the following locations and salinity zones: Tampa Bay, 18 ‰, 12 ‰, 6 ‰, Ruskin Inlet, and 0 ‰. Samples from two stations, the mouth of the river and 9 ‰, were collected on the first two sample dates (1/26/88 and 2/10/88) but were not sampled thereafter. Two other salinity zones, 12 ‰ and 6 ‰ replaced these two earlier stations. Results from the mouth and 9 ‰ will not be discussed in this report. Nutrient effects on carbon-14 uptake were determined at Tampa Bay, 12 ‰, 6 ‰ and 0 ‰.

Replicate surface samples were taken at each location. Subsamples for one cell count and species composition and carbon-14 uptake were taken from the first replicate after screening the water through a 153 um Nitex mesh net to remove detritus and larger zooplankton. The second cell count sample was taken from the second replicate after screening through the same Nitex mesh. Subsamples for counts and composition (100 ml) were preserved in the field with 2% (final concentration) neutralized formalin and kept dark and cold for transport to the laboratory. Subsamples for carbon-14 uptake were kept at field temperatures, in the dark, for transport to the laboratory, usually within two hours after collection. These samples were normally processed within 1-2 hours after return to the laboratory. The same set of replicate water samples was also used by others (SWFWMD, Brooksville Laboratory) for the determination of dissolved nutrients (nitrate, ammonium, phosphate, silicate) and particulate carbon, nitrogen and phosphorus. Methodology for these analyses can be found in their report. Chlorophyll-a concentrations were determined by FDNR, FMRI, St. Petersburg Laboratory, on each of the replicate surface samples. Chlorophyll was determined spectrophotometrically and concentrations were calculated using two methods: The Lorenzen method (Lorenzen, 1967) which accounts for the presence of phaeopigments, and the standard Jeffrey and Humphrey equations (Jeffrey and Humphrey, 1975) which does not. Both values have been used in this report. Seasonal chlorophyll cycles are depicted using the Jeffrey and Humphrey calculation since there were several sample dates which were not available from the Lorenzen calculation and because the Jeffrey and Humphrey values more closely reflected the seasonal variations in species counts. Chlorophyll concentration based on the Lorenzen method is, however, used in the calculation of the productivity index. Since carbon-14 uptake only occurs in living phytoplankton, the Lorenzen calculation, which corrects for chlorophyll degradation products associated with dead cells, yields an accurate representation of primary production. When Lorenzen chlorophyll values were not available, the productivity index was calculated using the Jeffrey and Humphrey value and will be noted in the text.

A single count for phytoplankton abundance and species composition was made on each of the replicate samples using a Sedgewick-Rafter counting chamber (1 ml volume) on an Olympus phase contrast microscope. Three to seven longitudinal paths were counted depending upon abundance; microflagellate counts are based on a single path. Thus 25-50% of the chamber volume was counted. Periodic determinations were made for the cumulative number of species identified by counting additional paths. The first path accounted for 50-68% of the total species while 83-95% were found within 3 paths. Cell size measurements were made on as many species as feasible. A total of 43 species or groups had shapes which would yield measurements applicable to geometric formulae for calculation of cell volume ( $\mu\text{m}^3$ ) and cell surface area ( $\mu\text{m}^2$ ). Cell size measurements were made microscopically and with an Image Analyzer System.

The effects of nitrogen and phosphorus additions on the potential photosynthetic rate, as measured by carbon-14 uptake, was assessed by partitioning the subsample of the first surface replicate into 60 ml, acid cleaned, glass bottles. Nitrogen (N) and Phosphorus (P), as nitrate and phosphate, were added to each set of duplicate bottles as follows: 0 N and P; 5  $\mu\text{M}$  N, 0 P; 0 N, 2  $\mu\text{M}$  P; 5  $\mu\text{M}$  N, 2  $\mu\text{M}$  P. In addition, 5  $\mu\text{M}$  N as ammonium was added to 2 additional dark bottles to determine the effect of N-enhanced dark carbon-14 uptake. An additional set of duplicate bottles from the 0 ‰ station were used as initial bottles (zero-time controls). This station was chosen since it normally had the highest particulate load. The initial bottles were filtered immediately after the addition of one microcurie of carbon-14 to determine the effect of adsorption and could also be used to correct the light bottle uptake rates if dark bottle uptake was high (Carpenter and Lively, 1980). However, dark bottle (without added ammonium) rates were always 10% or less of the light bottle uptake. Therefore all uptake rates were corrected using dark bottle counts. The initial bottle counts were not used. One microcurie of carbon-14 was added to the remaining bottles and they were incubated at  $300 \mu\text{E m}^{-2} \text{ s}^{-1}$  for 3 hours at the average ambient river temperature. Temperature was controlled by a circulating water bath. After incubation each bottle received 1 ml of a  $10^{-5}$  molar DCMU solution to stop further carbon uptake (Eppeley and

Renger, 1974; Bienfang and Szyper, 1981). Samples were then filtered onto 0.4  $\mu$ m Nuclepore filters, dissolved in a suitable tissue solvent, and after the addition of a scintillation cocktail, counted in a TM Analytic scintillation counter. Counts were corrected for quench and DPM (disintegrations per minute) calculated using a known standard and the external standard ratio method. Carbon uptake was then calculated by the appropriate equations in Strickland and Parsons (1972). Total carbonate in the sample, which is required for the calculation of carbon uptake, was determined on an Oceanography International Total Carbon Analyzer using triplicate 0.5 ml subsamples of the water used for measurement of carbon-14 uptake.

In addition to the above samples and analyses, light measurements were made at all stations. A Li-Cor quantum sensor was used to obtain irradiance readings of Photosynthetically Active Radiation (PAR, 400-700 nm) at the surface and approximately 0.5 m intervals throughout the water column with a final measurement as close to the bottom as practical. An extinction coefficient (k) was determined from these readings as the slope of an exponential curve fit of the data.

## RESULTS

### Station Locations

The Tampa Bay and Ruskin Inlet stations were the only fixed locations sampled during this study (see Fig. 1 and Table 1). The Tampa Bay station was located approximately 2.3 miles northeast of the mouth of the Little Manatee River, with Ruskin Inlet approximately 2.5 miles eastward from the mouth. All other river stations were sampled as regions of constant salinity, therefore their geographical locations varied throughout the year (Table 1). Thus, the 18 $^{\circ}$ /oo and 12 $^{\circ}$ /oo salinity zones were occasionally located outside the mouth of the river in Tampa Bay (Table 1). During periods of high river discharge (see Fig. 2; e.g. August 30 and September 8, 1988) all salinity zones were located at the mouth of the river or in Tampa Bay. All salinity zones showed a wide geographic variation in their respective locations (Table 1).

## Salinity

The seasonal cycle of salinity at Tampa Bay and Ruskin Inlet (Figs. 3 and 4), for those dates when salinities are available, is related to variations in river flow (see Fig. 2). Reduced salinity at both locations in March, July, August and September correspond to periods of increased river flow. Low river flow during spring and early summer yielded steadily increasing salinity at Ruskin Inlet with values greater than 20<sup>0</sup>/oo in late June and early July. The series of increases and decreases in salinity with a frequency of 2-4 week intervals at Ruskin Inlet during the fall and winter of 1988 and 1989 also correspond to variations in river flow. Salinities increase within the 2-week sampling time-frame which suggests relatively rapid mixing with higher salinity water from the river or Tampa Bay.

## Temperature

All locations show a similar seasonal cycle and range of temperature (Figs. 5 and 6). Rapid reduction in temperature during March and September correspond to periods of high river flow and increased runoff whereas the decline noted at all locations in November/December was not associated with riverflow. Winter minima at all locations was 14<sup>0</sup>C with summer maxima of approximately 30<sup>0</sup>C. The variation in temperature at any location for a particular sample date was generally within 1 to 2 degrees.

## Extinction Coefficients

Extinction coefficients are calculated as the slope of an exponential curve fit to the fraction of light reaching a given depth versus depth. Therefore, the slope of the line will be higher if light is attenuated rapidly. Clear, particle free water will have a low extinction coefficient. The values are given a negative numbers since they have a negative slope however, a high negative number (i.e. -2.5 vs. -0.5) indicates a high extinction coefficient and turbid water.

Annual mean values for the extinction coefficient of light attenuation in the water column increase from the lowest value in Tampa Bay to the highest at the fresh-water station at the head of the river (Table 2). The 18°/oo station had the lowest value for all river stations which corresponds to its ranking for total cell counts and chlorophyll concentration.

Seasonal cycles for light attenuation at all stations are seen in Figs. 7 to 12. All stations show considerable variation that can be related to both streamflow and phytoplankton biomass as chlorophyll. Streamflow alone does not control the extinction coefficient except in a general sense. Lower values (deeper light penetration) occur during the April to early July period of low, constant river flow (see Fig. 2) whereas values at the LMR stations increase during periods of high river flow (August - September). This is particularly true for the 0°/oo and Ruskin Inlet stations where maximum extinction coefficients coincide with periods of increased stream flow.

The annual mean extinction coefficients at 4 of the LMR stations can be related to the annual mean chlorophyll concentration (Fig. 13). The relationship fits an exponential curve with an  $r^2 = 0.99$ . Tampa Bay and Ruskin Inlet show annual mean coefficients that are lower for a given chlorophyll level than corresponding LMR stations. Extinction coefficients for Tampa Bay and Ruskin Inlet were plotted against the Jeffrey-Humphrey chlorophyll values for each sample date to determine if a relationship existed. A linear relationship gave the best fit between the two parameters for Tampa Bay (Fig. 14), however, the correlation coefficient of  $r^2 = 0.60$  (deleting the October 11 data point) suggests that factors other than phytoplankton biomass (e.g. sediment resuspension or water color) were contributing to the observed light attenuation. No relationship was found between the calculated extinction coefficient and chlorophyll at Ruskin Inlet (Fig. 15). The calculated line in that figure is the same as the seasonal average (1.91, Table 2). Therefore, as in Tampa Bay, factors other than phytoplankton biomass determine the extinction coefficient.

Based on the annual average extinction coefficient, the calculated depth of the 1% light level ranges from 1.59 to 4.0 meters for the 0°/oo and Tampa Bay stations, respectively. Since the bottom depth at most

all stations is 2.5m or less, all locations should have an autotrophic water column. The variation in percent of surface irradiance at each location for each sampling date confirms this (Table 3). Although the 0‰ location had less than 1% surface irradiance on 9 of 26 sample dates, the annual average extinction coefficient (Table 2) suggests that the water column at this salinity zone will be completely autotrophic.

#### Chlorophyll a seasonal cycles

All seasonal cycles of chlorophyll a (Chl) shown in Figs. 16 to 22, are based on the values determined by the Jeffrey-Humphrey calculation. In Tampa Bay the seasonal cycles can be characterized by winter minima (concentrations less than  $6 \text{ ug l}^{-1}$ , January to March, 1988 and November-December, 1989) with summer-fall maxima. Concentrations ranged from less than  $1 \text{ ug l}^{-1}$  to approximately  $34 \text{ ug l}^{-1}$  (Fig. 16). During the period of June through August, concentrations remained relatively constant at  $10 \text{ to } 15 \text{ ug l}^{-1}$ . The minimum that occurred on September 8, 1988 coincides with periods of high river flow (Fig. 2) and reduced salinity (Fig. 3). The September- October chlorophyll maximum corresponds to a period when river flow stabilized after the early September flow maximum. At this time a bloom of the blue-green alga, Schizothrix sp. occurred in Tampa Bay and the LMR (see below). The annual mean chlorophyll a concentration was  $7.46 \text{ ug l}^{-1}$  and ranked fifth among the 6 stations (Table 1).

Chlorophyll concentrations at 18 ‰ were generally lower than in Tampa Bay with a maximum of  $10 \text{ ug l}^{-1}$  occurring in May (Fig. 17). Winter values were similar in magnitude to Tampa Bay with relatively constant concentrations during June and July. The September-October maximum seen in Tampa Bay did not occur at this salinity zone although Schizothrix was present. This salinity zone was found in Tampa Bay from August 30, 1988 through December 8, 1988 and at the specific location of the Tampa Bay station on September 22 and October 11 (see Table 1). Therefore, chlorophyll values assigned to Tampa Bay for these dates in Fig. 16 can also be interpreted as occurring at the 18‰ salinity zone. However, they were not included in calculating the annual

averages listed in Table 2. The annual mean concentration of  $4.27 \text{ ug l}^{-1}$  was the lowest of the 6 stations sampled (Table 2).

At the  $12 \text{ }^{\circ}\text{oo}$  salinity zone, chlorophyll concentrations displayed a cycle that differed from Tampa Bay and  $18 \text{ }^{\circ}\text{oo}$  with a maximum in early July (Fig. 18). Winter minima were followed by relatively constant levels ( $10 \text{ ug l}^{-1}$ ) during April and May. A series of short-lived blooms occurred during August and September. This salinity zone also occurred at the specific location of the Tampa Bay station on September 8, 1988 (see Table 1). Schizothrix was present at this salinity on that date. Chlorophyll values assigned to Tampa Bay can therefore be interpreted as occurring at this salinity zone. The annual average chlorophyll concentration at this salinity zone was  $9.41 \text{ ug l}^{-1}$  and ranked fourth among the 6 stations (Table 2).

The seasonal cycle of chlorophyll at  $6 \text{ }^{\circ}\text{oo}$  (Fig. 19) differs from all other stations with the exception of the August-September minimum that corresponds to the period of high river flow. This salinity zone can best be characterized by a series of blooms with a periodicity that varies from semi-monthly to monthly. The annual maximum occurred in May when the phytoplankton community was dominated by microflagellates and a small Cyclotella sp.. This species was also found at the  $0 \text{ }^{\circ}\text{oo}$  salinity zone on this date. The annual average chlorophyll concentration was  $13.63 \text{ ug l}^{-1}$ , which ranked third highest of the 6 stations (Table 2).

Chlorophyll concentrations at Ruskin Inlet (Fig. 20) also display a cycle which indicates that a series of blooms occurred with about a 1 month frequency. The August-September flushing event also reduced chlorophyll levels at this station. Chlorophyll concentration increased rapidly after the flushing event to the annual maximum of  $50 \text{ ug l}^{-1}$  in early November. This peak occurred about one month later than in Tampa Bay. Chlorophyll concentration fluctuated between 10 and  $25 \text{ ug l}^{-1}$  during the rest of the year with an annual average of  $17.79 \text{ ug l}^{-1}$  (Table 2).

The  $0 \text{ }^{\circ}\text{oo}$  salinity zone had the greatest annual range of chlorophyll concentrations, varying from approximately  $1 \text{ ug l}^{-1}$  during the August-September flushing event, to a maximum exceeding  $60 \text{ ug l}^{-1}$  in late October when river flow stabilized (Fig. 21). Minimum values were found in January 1988 and 1989 and during August-September 1988. The



annual average was  $18.32 \text{ ug l}^{-1}$  which was the highest of all LMR stations and Tampa Bay (Table 2). The seasonal chlorophyll cycle at this salinity zone can also be described as a series of short-lived blooms with concentrations seldom falling below  $10 \text{ ug l}^{-1}$ .

Chlorophyll concentrations from the Wimauma station on the Little Manatee River are included in this report for comparison with the  $0^{\circ}/\text{oo}$  and other Little Manatee River salinity zones (Fig.22). They were obtained from Mr. Sid Flannery, SWFWMD. Although there are a considerable number of missing values, the general trend at Wimauma appears to be a series of short-lived blooms with approximately a monthly periodicity. The outstanding feature of the seasonal cycle at this location is the low chlorophyll concentrations. The maximum that occurred in late July was less than  $8 \text{ ug l}^{-1}$  with most other values less than  $4 \text{ ug l}^{-1}$ . Therefore, the chlorophyll concentration at Wimauma was considerably less than that recorded at the freshwater salinity zone ( $0^{\circ}/\text{oo}$ ) and at the  $18^{\circ}/\text{oo}$  salinity zone; the region with the lowest annual average for the River. Elevated chlorophyll concentrations at the  $0^{\circ}/\text{oo}$  zone therefore were the result of phytoplankton growth between Wimauma and  $0^{\circ}/\text{oo}$  rather than transport from Wimauma, which is considerably east of the  $0^{\circ}/\text{oo}$  salinity zone.

In general terms, the period of increased river flow in August and early September reduced chlorophyll concentrations at all LMR stations and Tampa Bay to their annual minimum. Major blooms developed at 3 stations (Tampa Bay, Ruskin Inlet and  $0^{\circ}/\text{oo}$ ) shortly thereafter, during a period of decreasing river flow (Fig. 2) which should allow restablization of the water column. At all other times of the year, the seasonal cycle of chlorophyll at all LMR stations can be characterized as a series of blooms with a semi-monthly to monthly frequency that show little relationship to river flow characteristics.

#### Numerical abundance and composition

##### Tampa Bay

Elevated numerical abundance in January and February, 1988 (Fig. 23) was not reflected in the seasonal chlorophyll cycle (Fig. 16). Diatoms, primarily Skeletonema costatum, dominated during this period

and into March (Fig. 24 and 25). Low and essentially constant total population density occurred during the period of low river flow from March through mid-June (Fig. 22) which did not reflect the variable and elevated chlorophyll levels measured during that time period. The bloom that occurred at the end of June was dominated by the diatom Thalassionema nitzschoides, while S. costatum and Nitzschia pungens, a chain forming pennate diatom, were dominant during the July 28, 1988 peak of abundance. Diatom abundance decrease during spring and displayed an inverse cycle with microflagellate populations (Fig. 24). The two groups contributed greater than 80% of the total population throughout most of the year. Exceptions to this generalization occurred during the period of high river flow during August-September, 1988 and September-October when the blue-green alga, Schizothrix sp. became the numerical dominant (Fig. 24 and 26). This latter bloom coincided with the annual chlorophyll maximum (Fig. 16). Schizothrix first appeared in early August when river flow increased (Fig. 2) and salinity decreased (Fig. 3) and persisted to the first November sampling. This blue-green alga was present throughout Tampa Bay during this period (COT, pers. comm.)

Dinoflagellate populations were present throughout the year (Fig. 27) but never represented more than 5% of the total abundance. Prorocentrum micans was the major contributor to the total population during March and April, 1988. Chlorophytes were rarely present in Tampa Bay although five species were noted during the August-September period of high river flow and reduced salinity (Fig. 27). Other groups (Fig. 28) were minor components of the phytoplankton community.

#### 18<sup>0</sup>/oo Salinity Zone

The seasonal cycle of phytoplankton abundance at this salinity zone is distinct from Tampa Bay and all other river stations. Total abundance at this salinity zone ranked sixth (Table 2) with an annual mean of 2808.8 cell ml<sup>-1</sup> which, as with chlorophyll, was the lowest rank for a river location. Peaks in abundance occurred in March and July (Fig. 29). The maximum in September reflects counts obtained for Tampa Bay when the 18<sup>0</sup>/oo salinity zone was located at this station. No

chlorophyll data is available for the March date, while the July maximum corresponds to a slight increase in chlorophyll. Both peaks in numerical abundance correspond to increases in the population of microflagellates (Fig. 30), which accounted for 94% and 88% of the total populations in March and July, respectively. With the exception of the January-March, 1988 period when diatoms contributed more than 70% of the total numerical abundance, microflagellates dominated numerically at this salinity zone (Fig. 30). Two short-lived diatom pulses occurred in May and July, respectively (Fig. 30). The May increase was due to Ceratulina bergonii while S. costatum was the most abundant diatom in July (see Fig. 25). Dinoflagellate populations increased in March-April but contributed less than 4% of the total abundance (Fig. 31). Two Peridinium species, Peridinium sp. and Peridinium aciculiferum, along with Prorocentrum micans were responsible for this peak, although cell counts were only in the range of 20-40 cells ml<sup>-1</sup>. The August-September bloom of the blue-green alga, Schizothrix sp. was noted at this station and accounted for 47% of the total in September with populations of more than 10,000 filaments ml<sup>-1</sup> (Fig. 26 and 30). Populations of Chlorophytes were greater and occurred more frequently than in Tampa Bay but never accounted for more than 1.2% of the total population (Fig. 31). Others, such as silicoflagellates and Pyramimonas were present occasionally but never contributed more than a few percent of the total numerical abundance (Fig. 32).

#### 12<sup>0</sup>/oo Salinity Zone

The annual cycle of total phytoplankton abundance at the 12<sup>0</sup>/oo salinity zone can be characterized as a series of peaks that vary widely in magnitude over the two week sampling interval. From March, 1988 through June, there is little correspondance with the annual chlorophyll cycle (compare Figs. 18 and 33). Following the July peak of maximum abundance however, the fluctuating cycle is reflected in chlorophyll concentration. This July maximum can be attributed to a bloom of an unknown naked dinoflagellate which, unfortunately, burst upon preservation and could not be identified although it contributed 26.5% of the total cell numerical abundance (Fig. 35).

Microflagellates dominated this station numerically throughout the year (Fig. 34) with the exception of the October 11 sampling date. In September, Schizothrix sp. was the numerical dominant (Figs. 26 and 35) while populations of three diatom species, S. costatum, Chaetoceros subtilus and Leptocylindrus minimus, combined to account for 60% of the total abundance on October 11 (Figs. 25 and 34). However, this October increase was not characteristic of this station where diatom populations contributed less than 30% of the total community abundance during the annual cycle. Chlorophytes increased in their frequency of occurrence (Fig. 36) but were also a minor component of the community. The maximum in Chlorophytes as a fraction of the total population that occurred in early March was the result of 6 species being present. The phagotrophic Euglenoid flagellate, Eutreptia sp. was the dominant chlorophyte on that date with populations of 28 cells ml<sup>-1</sup>.

Total numerical abundance at this salinity zone ranked fifth lowest of the six stations sampled with an annual average of 3260.8 cells ml<sup>-1</sup> (Table 2).

#### 6°/oo Salinity Zone

Sampling at this station for cell counts began in April. However, the April 6 sample was not counted in request for a bottom sample count at 12°/oo and the April 20 samples from all stations were lost as a result of incomplete preservation. Therefore the annual cycle of phytoplankton abundance for this salinity zone begins in May, 1988.

The general trends of total numerical abundance (Fig. 37) resemble the annual chlorophyll cycle (Fig. 19) with a maximum in May, lower population density during late summer (August-September) and a series of peaks in October, November, and January, 1989. Two diatoms, S. costatum and S. costatum combined with Thalassiosira pseudonana were the primary diatom species responsible for the October and December peaks, respectively (Fig. 38 and 39), although the cell count for the December peak was less than 1000 cell ml<sup>-1</sup>. However, microflagellate populations were the numerical dominants throughout most of the year at this salinity zone (Fig. 38). Peridinium aciculiferum, was the species responsible for the increased dinoflagellate contribution to the

community during June and July (Fig. 40). Schizothrix was also found at this salinity zone with a population maximum at the end of September (Fig. 40 and 41), but represented a lower percentage of the total abundance than at higher salinity zones. On September 8 this salinity zone was located just outside the mouth of the river but by the next sample date, September 22, it was located 4.1 miles upstream (Table 1). Schizothrix was first found on September 8 followed by a population increase on September 22. The seed population could have been entrained in this lower salinity water and with subsequent growth between September 8 and 22, produced the elevated population. Alternately, since the bloom started in Tampa Bay following the major salinity reduction in August, and it was found at all higher salinity zones, entrainment from Tampa Bay by tidal mixing is more likely.

Fresh water species of chlorophytes showed an increased frequency of occurrence at this salinity zone although their contribution to the total community abundance was still less than 2.5% (Fig. 42). Peaks in their percent contribution to the total community in May and mid-June were due to two different Scenedesmus species, while a mixture of 6 species contributed to the August peak. Akistrodesmus falcatus was responsible for the October maximum and Euglenoid flagellates were prevalent in December-January, 1989. Transport of these freshwater species from upstream regions probably occurred. Although they were identified in preserved samples, they had the appearance of being live cells at the time of collection. The annual average was 4314 cells ml<sup>-1</sup> at this salinity zone ranked fourth in total abundance.

#### 0‰ Salinity Zone

Generally, the annual cycles of total phytoplankton numerical abundance and chlorophyll concentration showed similar trends at this fresh-water station (Figs. 43 and 21). There is a relative consistency in total numbers throughout the year with a major bloom during the first sampling in October which corresponds to elevated populations of the diatom S. costatum (population density greater than 30,000 cells ml<sup>-1</sup>, Fig. 39). Populations of two Cyclotella spp. were responsible for the continued dominance of diatoms during the second October sample (Fig.

44) which coincided with the chlorophyll maximum noted on that date (Fig. 21). Microflagellates represented a greater proportion of the community from March through September and either co-dominated or showed an inverse relationship with diatom abundance at other times (Fig. 44).

Chlorophytes represented a greater percentage of the total population at this fresh-water station than at all other stations with a maximum of approximately 7% in late September (Fig. 45). Populations of Akistrodesmus, at 40 to 50 cells  $\text{ml}^{-1}$ , were the most abundant representatives of the Chlorophyta on this sampling date. The blue-green algae, Schizothrix sp., did not penetrate to this salinity zone. Blue-green algae (e.g. Merismopedia sp.) were relatively rare but always present (Fig. 45). Other groups (Fig. 46) made up less than 1% of the total phytoplankton community. Despite the major October bloom with population levels of greater than 35,000 cells  $\text{ml}^{-1}$ , the annual average for this station was 4712.1 cells  $\text{ml}^{-1}$  which ranked third among the six stations (Table 2).

#### Ruskin Inlet

The annual cycle of abundance at Ruskin Inlet closely resembles that of 0°/oo with a relative consistency in total numbers throughout the year and a major peak in abundance in early October (Fig. 47). The cycle of abundance did not entirely reflect the seasonal cycle in chlorophyll concentration (see Fig. 20). Maximum abundance occurred on October 11 whereas the chlorophyll maximum occurred on November 7. Other chlorophyll peaks in February and April are mirrored by cell counts but the July chlorophyll peak occurs one sample date after a peak in abundance. No good explanation can be offered for this dissimilarity.

The annual average of total phytoplankton was the highest of all stations at 9391.1 cells  $\text{ml}^{-1}$ , almost double that noted for the fresh-water station and 3-fold greater than at 18°/oo (Table 2). Microflagellates and diatoms show a complex seasonal pattern of numerical dominance with inverse shifts at about monthly intervals (Fig. 48).

Skeletonema costatum occurred throughout the year at this station and was responsible for the October maximum with populations that exceeded 80,000 cells  $\text{ml}^{-1}$  (Figs. 39 and 48). This species, combined with a small unidentified Thalassiosira species, was responsible for the continued numerical dominance of diatoms through November (Fig. 48). The S. costatum bloom on October 11 did not coincide with the annual chlorophyll maximum on November 7. Although S. costatum was the numerical dominant on November 7, its population density had decreased to 12,000 cells  $\text{ml}^{-1}$ . This S. costatum bloom did not coincide with August-October Schizothrix bloom in Tampa Bay. This blue-green alga occurred in Ruskin Inlet from August through September (Figs. 41 and 50) but was reduced to trace population levels at the time of the S. costatum bloom.

Of particular interest is the almost continuous presence of chlorophyte populations, especially the Euglenoid flagellates, Eutreptia and Euglena spp. which are phagotrophic and associated with high organic particle loads. These two genera contributed approximately 20% of the total abundance during January, 1988, July, and January, 1989 (Figs. 49 and 52). Similarly, dinoflagellate populations were continuously present in Ruskin Inlet, although at low population levels. The dinoflagellate bloom in early February, which accounted for almost 80% of the total abundance (Fig. 49) was due to a naked species which burst upon preservation. A bloom of a species which showed the same characteristics upon preservation occurred at the 12‰ salinity zone in July, 1988. The minor peak in dinoflagellate abundance in April (Fig. 49) resulted from the increased abundance of Prorocentrum minimum. This species was present at Ruskin Inlet and all other stations at low (less than 50 cell  $\text{ml}^{-1}$ ) population levels (Figs. 53 and 54). It is a known red-tide species in other areas (e.g. Chesapeake Bay) and may be responsible for fish-kills. There have been anecdotal reports of red-water in Ruskin Inlet, but our sampling program did not appear to coincide with a bloom of this species.

Although Schizothrix sp. was found at Ruskin Inlet during the August-September blue-green bloom period (Fig. 41), it was not the numerical dominant for this group during the August peak noted in Fig. 50. Merismopedia punctata was the major contributor in August, while

Schizothrix and Nostoc sp. (trichomes) combined to contribute 4% of the total population density in September. The population density of Schizothrix at Ruskin Inlet was considerably lower than at any other River salinity zone (compare Figs. 26 and 41). Although the presence of this species suggests that transport from Tampa Bay occurred, the low population levels indicate that competition from other species or environmental conditions were not favorable for growth.

#### Phytoplankton community composition in relation to salinity

Variation in the phytoplankton community composition, as indicated by changes in the contribution made by several groups to the total abundance, at the various fixed stations and salinity zones of the LMR for each sampling date is depicted in Figs. 55 to 79. Several generalities are evident. Diatoms and microflagellates contribute a greater percentage of the total phytoplankton abundance throughout the year than all other groups combined. The only exception to the dominance of these two groups occurred during September, 1988 at all locations except 0<sup>0</sup>/oo when the Schizothrix bloom occurred in Tampa Bay and the LMR. Microflagellates become increasingly important at all river stations in spring and are the numerical dominants throughout the summer, and with the exception of one or two stations on varying sample days, they are the numerically dominant group during the August-October Schizothrix bloom.

It is difficult to discern a consistent pattern in total phytoplankton counts from Tampa Bay up-river to zero salinity (Figs. 80,81), although the average total counts for each station and salinity zone (Table 2) suggest a trend with highest populations in the Bay, reduced abundance at 18<sup>0</sup>/oo and gradually increasing abundance upstream. Ruskin Inlet, although it had the highest annual mean total count is not included because it is a eutrophied arm of the river. Diatom populations were more abundant in Tampa Bay than at any of the LMR locations (Figs. 82,83) comprising an annual average of 44.8% of the total phytoplankton counts. Diatom populations varied from a minimum of 17.5% of the total counts at 12<sup>0</sup>/oo to 31.4% at 0<sup>0</sup>/oo. Microflagellates were therefore the numerically dominant group at all river locations



with annual means that ranged from 64.8% of the total counts at 0<sup>0</sup>/oo to 76.1% at 12<sup>0</sup>/oo. In Tampa Bay, microflagellates comprised an annual average of 43.9% of the total count. These values are reflected in Figs. 84 and 85 and in Table 4 which summarizes the annual mean abundance for species and groups that occurred on 13 or more sampling dates. The annual mean, median and range of abundance for all species identified, irregardless of location is presented in Appendix Table 3. Total counts for each major group arranged by location for each sampling date are summarized in Appendix Table 4 and the same information arranged by sampling date for each location is in Appendix Table 5. The combination of diatoms and microflagellates represented a range of 86.6% to 96.2 % of the total phytoplankton abundance at all stations. Several diatom species occurred at all stations; Skeletonema costatum, Nitzschia closterium, Nitzschia longissima, Thalassiosira pseudonana and Chaetoceros subtilus (Tables 4,5 and Figs. 25,39,92 and 93). S. costatum and the two Nitzschia spp. show a broad euryhaline distribution. The presence of S. costatum at 0<sup>0</sup>/oo is unusual as is discussed later. Chaetoceros subtilus (Figs. 92,93) however, occurred more frequently at intermeadiate salinities (12 and 6<sup>0</sup>/oo, Table 5) than at higher salinities. Diatoms such as Chaetoceros socialis, Leptocylindrus minimus, Cerataulina bergonii, Asterionella glacialis and Thalassionema nitzschoides occurred more frequently at higher salinities (Table 5).

Although dinoflagellates (Figs. 86 and 87) were never abundant in the Bay or the LMR, several species were present at all locations. Prorocentrum minimum, Peridinium aciculiferum and Gymnodinium spp. were found at all salinity zones (Table 5) while Gonyaulax occurred at all locations except 0<sup>0</sup>/oo. P. minimum was found most frequently in Ruskin Inlet and Tampa Bay (Table 5) although the highest populations were found in Ruskin Inlet (Figs. 53 and 54). P. aciculiferum occurred within the river and Ruskin Inlet with greater frequency than Tampa Bay (Table 5) suggesting a preference for low to intermediate salinities.

The seasonal abundance of blue-green algae is governed more by the August-October Schizothrix bloom than by salinity (see Figs. 26,41 and 88,89). As noted above, the August and September maxima were the result

of a bloom of this species. Only one species, Nostoc sp., occurred at all location (Table 5) but only for a limited period.

Chlorophytes, as one might expect, occurred more frequently at the lower salinity zones (Table 5, Figs. 90,91). Scenedesmus quadricauda and Schroederia setigera were occasionaly found in Tampa Bay after periods of elevated river flow. Eutreptia spp. and other euglenoids were most common in Ruskin Inlet (see Figs. 51,52, Tables 4,5). These species are indicators of organic rich areas and are associated with eutrophication. They rarely occurred at other locations.

#### Similarity Index

This index is based on the presence or absence of a species in the combined replicate counts for each station. The community in Tampa Bay for any given sampling date was compared to all LMR stations (Table 6). Although there is considerable seasonal variation in similarity at a given station, the general trend is for a decrease in the annual average similarity from the mouth to the head of the river. The fact that 20% of the species found in Tampa Bay also occurred at 0<sup>o</sup>/oo salinity with a fair degree of consistency indicates the euryhaline nature of the community in Tampa Bay and suggests that transport of Bay water with accompanying cells to the head of the river occurs regularly. Although 34 of the 204 species or groups identified were found at 0<sup>o</sup>/oo and Tampa Bay (see Table 5), most were blue-greens and chlorophytes; species characteristic of fresh water habitats. Estuarine diatoms and dinoflagellates such as N. closterium, N. longissima, S. costatum, C. subtilus and P. minimum, which are commonly found at higher salinities, occurred at the freshwater location which suggests up-river transport.

Ruskin Inlet displays as much similarity with Tampa Bay as does the 12<sup>o</sup>/oo salinity zone with an annual average of 0.36. The salinity at Ruskin Inlet varied considerably throughout the annual cycle (Fig. 4). Similarity index values averaged greater than 0.3 at Ruskin Inlet when salinity was greater than 11<sup>o</sup>/oo; which also emphasizes the euryhaline nature of the phytoplankton community.

## Diversity Index

The Shannon-Weaver diversity index is a measure of the species richness (number of species) and the proportion of the total community that species represents. Therefore, it can also be viewed as an index of the evenness of distribution of populations within a community. Low values will be calculated if few species are present or if a few species contribute the bulk of the total population abundance, i.e., a relatively monospecific bloom.

The seasonal variation in diversity is high at all stations and exhibits a wide range (from 0.0 at Tampa Bay on June 15 when only one species was identified to genus to -4.599 in Ruskin Inlet on August 10, Table 7). The annual means indicate increasing diversity and evenness of distribution among species in the upstream direction. However, the annual variability at each river station suggests that there would be no significant difference between the annual means for each river station. The annual means for both Tampa Bay and Ruskin Inlet are lower than for the other river stations which reflect the periodic blooms dominated by one or two species that occur at these two stations. Although both have high species richness (i.e. a high number of species present), the dominance in the community by one or two species, with others present in low relative abundance, yields a low Shannon-Weaver index.

## Cell Volume and Cell Surface Area

Cell size measurements were made on a total of 43 species which could be approximated by geometric shapes from which volume and surface area could be calculated. An average cell size, based on measurements of the same species from different sampling dates, was used in all calculations of total cell volume and surface area. Although cell size for a given species did vary between samples, the variation was small relative to differences in total abundance. Since the objective of this part of the study was to determine if the numerical dominant also dominated the community with respect to cell volume and surface area, parameters which have ecological and physiological relevance, the average cell size was used in all calculations.

Total cell volume and total cell surface area for all available species is presented in a table in Appendix 1. Since data were not available for all species counted at each station, total cell volume and surface area could not be calculated. Therefore, since microflagellates and the diatom, Skeletonema costatum, were the numerical dominants at essentially all stations for most of the year (see Table 4 and Appendix 1), they were chosen to highlight the annual cycle of cell volume (see Figs. 94 to 105).

In Tampa Bay (Figs. 94,95), microflagellates and S. costatum co-occurred on 20 of 26 sampling dates. Total cell volume for microflagellates was greater than for S. costatum on only two of those dates (April 6 and June 29). On April 6, the diatoms Chaetoceros neogracile and Ceratulina bergonii and several minor species dominated the community with respect to cell volume ( $3,170,283 \text{ um}^3$ ). Similarly, on June 29, the diatom Thalassionema nitzschoides was both the numerical and cell volume dominant ( $17,474,836 \text{ um}^3$ ).

At  $18^\circ\text{oo}$ , microflagellates and S. costatum co-occurred on 20 of 26 sampling dates with microflagellate cell volume greater than S. costatum on 4 dates (Appendix 1). However, with the exception of the December 8 sampling date, total diatom cell volume was greater than microflagellates (Figs. 96,97). The same result was obtained for the  $12^\circ\text{oo}$  station (see Figs. 98,99; Appendix 1) with May 18 being the only date when microflagellates dominated numerically and by cell volume.

S. costatum and microflagellates co-occurred on 19 sampling dates at the  $6^\circ\text{oo}$  salinity zone with 9 dates when microflagellate cell volume was greater than S. costatum (Figs. 100,101). As at other stations, total diatom cell volume reduced the times when microflagellates dominated numerically and volumetrically to 3. Although the overall abundance of microflagellates was greater at Ruskin Inlet than at any other LMR station, this group was never dominant with respect to cell volume (Figs. 102,103). At  $0^\circ\text{oo}$ , microflagellates and S. costatum co-occurred on 18 of 26 sampling dates yet flagellates dominated volumetrically on only one of those dates (see Figs. 104,105). However, there is a question of the viability of S. costatum at this location. Cells always had a dense appearance without distinct chloroplasts and the intracellular space was much reduced. A definitive determination of

viability cannot be made, so for the purpose of this study we will only report this species presence and contribution to the community with no assumption of viability.

#### PRODUCTION AND NUTRIENT ADDITION EXPERIMENTS

Volumetric Production Rates ( $\text{mgC m}^{-3} \text{ hr}^{-1}$ ) and Productivity Index ( $\text{mgC mgChl}^{-1} \text{ hr}^{-1}$ )

Volumetric productivity and the productivity index (PI) are based on the ON,OP replicate samples incubated at approximately  $300 \text{ uE m}^{-2} \text{ s}^{-1}$  at the temperature at which samples were collected. The irradiance value was chosen to yield maximum production rates without photoinhibition responses. With a surface irradiance of  $1900 \text{ uE m}^{-2} \text{ s}^{-1}$  this value is approximately 16% of the surface intensity. Based on the annual average extinction coefficients (Table 2), an irradiance of  $300 \text{ uE m}^{-2} \text{ s}^{-1}$  would be found between 0.6m and 1.6m at the 0°/00 and Tampa Bay stations, respectively. It should, therefore be close to an average water column irradiance. Since light was constant for all sampling dates, productivity and the PI should only vary with temperature, biomass or nutrient availability.

#### Tampa Bay

Volumetric production rates in Tampa Bay were generally low throughout the Fall and Spring with values of  $50 \text{ mgC m}^{-3} \text{ hr}^{-1}$  or less (Fig. 106). From June through August, production increased dramatically with elevated rates all greater than  $100 \text{ mgC m}^{-3} \text{ hr}^{-1}$ . The maximum in early June corresponds to a period of low cell counts (see Fig. 23) with microflagellates numerically dominant (see Fig. 24) and minor diatom populations. Chlorophyll concentrations were elevated from the previous sample date (see Fig. 16). The annual minimum occurred in early September during the period of maximum river flow (Fig. 2). The peak that occurred on the last sample date in September is displaced by one sample period from the October chlorophyll maximum but occurs when the community was dominated by the blue-green alga, Schizothrix. The annual

average for production at the Tampa Bay station was  $89.88 \text{ mgC m}^{-3} \text{ hr}^{-1}$  which ranked second of 4 stations (Table 2).

The PI displays considerable seasonal variation, with values that range from less than 1 to greater than 20 (Fig. 107). Lowest values occur during April and May when cell counts were low and chlorophyll concentrations were highly variable. Microflagellates numerically dominated the community with diatoms comprising less than 20% of the total abundance. However, for the remainder of the year, the PI was greater than 5 which can be considered indicative of nutrient replete populations (Curl and Small, 1965, Eppley et al., 1973, Eppley, 1981). From September through January, 1989, the PI was relatively constant despite wide variations in chlorophyll and total abundance.

#### $18^{\circ}/\text{oo}$ Salinity Zone

Volumetric production rates at this salinity zone were relatively constant throughout the year (Fig. 106). Rates were always less than  $100 \text{ mgC m}^{-3} \text{ hr}^{-1}$  with an annual minimum during the September period of high river flow. In September and October, when the  $18^{\circ}/\text{oo}$  salinity zone was located in Tampa Bay, production rates for the Bay were assumed to be equivalent to  $18^{\circ}/\text{oo}$ .

The consistency of production rates at this station correspond to its low ranking in total population abundance and chlorophyll concentration. With an annual average of  $50.89 \text{ mgC m}^{-3} \text{ hr}^{-1}$  it was again the lowest ranked station (Table 2).

However, populations at this station displayed the highest PI (see Table 1) which also showed a high degree of consistency (Fig. 107) despite a four-fold variation in chlorophyll concentration and wide fluctuation in total abundance. All PI values at this salinity zone were above 5, with the majority above 10, which is indicative of nutrient sufficient populations.

#### $12^{\circ}/\text{oo}$ Salinity Zone

As at  $18^{\circ}/\text{oo}$ , the annual cycle at this salinity zone displays less variation than in Tampa Bay with volumetric production rates generally

less than  $100 \text{ mgC m}^{-3} \text{ hr}^{-1}$  (Fig. 108). However values greater than 100 occur from June through early August. The maximum occurs during the first sample in July and corresponds to the chlorophyll maximum and the maximum in total abundance. There is no general relationship with total abundance, however, since this station displayed the greatest sample to sample variation of any location (see Fig. 33).

The PI is essentially constant throughout the year at approximately  $10 \text{ mgC mgChl}^{-1} \text{ hr}^{-1}$ . The essentially constant PI for this station suggests that production was strictly a function of biomass with no nutrient limitation.

#### $0^{\circ}/\text{oo}$ Salinity Zone

Volumetric production rates at this salinity zone display a high degree of annual variation (Fig. 108) reflecting a similar degree of variability in chlorophyll concentration (see Fig. 21). Decreasing production during July, August and September reflects low cell counts (see Fig. 43), low chlorophyll concentrations and increasing stream flow. The October production maximum corresponds to the chlorophyll maximum and a diatom bloom of the genus Cyclotella.

The PI displays the same consistency noted for the previous salinity zone (Fig. 109) although values are lower during fall and winter. The maximum value of 17 occurred on September 22, when chlorophyll and cell counts were low and microflagellates contributed 80% of the total abundance.

This salinity zone had the highest mean annual production rate ( $122.25 \text{ mgC m}^{-3} \text{ hr}^{-1}$ ) but ranked lowest with a PI of  $8.79 \text{ mgC mgChl}^{-1} \text{ hr}^{-1}$  (Table 2).

#### Effects of Nitrogen and Phosphorus Additions on Short-term Photosynthesis

Potential nutrient limitation of short-term photosynthesis (3 hr. incubations) and potential nitrogen limitation of the community was assessed in two ways: 1. Nitrate and phosphate alone and in combination were added to replicate bottles incubated in the light at  $300 \text{ uE m}^{-2} \text{ s}^{-1}$

to assess if stimulation, inhibition or no effect occurred during photosynthesis and, 2. An ammonium addition was made to replicate dark bottles to determine the effect on carbon-14 uptake in the dark. The latter effect was assessed by calculating the ratio of CPM (counts per minute) in the dark bottles (D) with (+) and without (-) ammonium and by calculating the actual production rate in the ammonium addition bottles over and above the standard dark bottle correction (Table 8;  $D+/D- = \text{CPM}/\text{CPM}$ ;  $V_{D+} - V_{D-} = \text{mgC m}^{-3} \text{ hr}^{-1}$ ). Addition of ammonium to nitrogen limited populations should enhance dark carbon-14 uptake (Morris et al., 1971; Elrifí and Turpin, 1987).

The effect of nitrate and phosphorus additions on photosynthesis is shown in Figs. 110 to 121 for the four stations at which carbon-14 uptake measurements were made. The results are expressed as the difference in the average photosynthesis rates between the bottles receiving a nutrient addition and those receiving no addition (ON, OP). Thus a positive value indicates an enhancement of photosynthesis as a result of the nutrient addition while a negative value indicates inhibition. The productivity index rather than the volumetric production rate was used since the differences will be smaller numbers which reduces the range of values used in the plots. Since all photosynthesis measurements were only done in duplicate, statistical analyses were not performed. However, if the difference between the PI index for any nutrient addition and the index for ONOP was equal to or greater than 2, the range of each replicate was examined. If the range for each treatment overlapped, then I inferred there was no difference between treatments.

Additions of nitrate and phosphate had little effect on photosynthesis at any station. Differences between samples with and without nutrient additions were generally less than 2. There were however some specific sampling dates at each station where the ranges of the paired bottles did not overlap. For example, in Tampa Bay the addition of nitrate alone reduced photosynthesis in the July 28 sample (Fig. 110), while the addition of phosphate alone or in combination had no effect (Figs. 111, 112). Although there appears to be an enhancement of photosynthesis by the addition of nitrate and phosphate to the June 15 sample (Fig. 110), the ranges of both sets of replicates overlapped



considerably. Phosphate alone reduced photosynthesis in the Feb. 10 sample (Fig. 111), but no other effect was noted. The addition of nitrate and phosphate in combination produced a trend for reduction in photosynthesis throughout the year (Fig. 112), however, none of the differences were significant.

At 18<sup>0</sup>/oo the addition of nitrate enhanced photosynthesis only in the July 14 sample (Fig. 113), whereas the addition of phosphate alone and in combination with nitrate enhanced carbon-14 uptake in samples from June 29 through August 10 (Figs. 114,115). This is somewhat enigmatic since phosphate concentrations at this station were always present in excess with concentrations never less than 45 ug l<sup>-1</sup> (4.7 ug at l<sup>-1</sup>).

The degree of stimulation/reduction in samples from the 12<sup>0</sup>/oo salinity zone showed a mixed seasonal response. Nitrate addition yielded a significant enhancement only in the January 24, 1989 sample (Fig. 116). Phosphate additions enhanced photosynthesis in March, July and January, 1989 while causing a significant reduction in August (Fig. 117). When added in combination with nitrate, phosphate only enhanced photosynthesis in October (Fig. 118).

At 0<sup>0</sup>/oo, the addition of nitrate alone, phosphate alone and in combination reduced photosynthesis significantly on only two dates, April 18 and August 30 (Figs. 119,120,121). Neither inorganic nitrogen or phosphorus concentrations were depleted in the water column on either date and the August sample coincided with minimum chlorophyll concentrations and low numerical abundance.

Ammonium additions enhanced dark carbon-14 uptake on a majority of sampling dates at all stations based on the calculation of  $V_{D+} - V_{D-}$  (Figs. 122 to 125). However, the ratio of  $D+/D-$  exceeded 2 only on 2 occasions (July 28 at 18<sup>0</sup>/oo and April 18 at 12<sup>0</sup>/oo, Table 4) with almost all values falling in the range of 1 to 2. Therefore the degree of enhanced dark carbon-14 uptake following nitrogen addition must be considered as a trend indicative of nutrient sufficient to borderline nitrogen limitation.

## DISCUSSION

### Community composition and abundance

The phytoplankton community in the Little Manatee River and at the Tampa Bay station consists of a seasonally varying mixture of representative species of diatoms, dinoflagellates, microflagellates and chlorophytes with a sporadic occurrence of blue-green algae. A total of 91 taxa of diatoms (some only to genera), 35 dinoflagellate taxa, 57 chlorophytes, 6 blue-green alga genera, 3 silicoflagellates and several representatives of other diverse microalgal groups were identified and counted. Microflagellates, which consisted of a diverse group of unidentified species, were not identified to a specific taxon but were counted as a group. The number of taxa are comparable to the extensive list of phytoplankton summarized by Steidinger and Gardiner (1985, see their Table 1). Similarly, total abundance at the LMR Tampa Bay station is comparable to that reported by the City of Tampa (COT, 1981) for their Station 13 in mid-Tampa Bay and exhibits a similar seasonal cycle with lower population density during March, April and May and a series of blooms throughout the summer, fall and winter. Seasonal average cell counts for the LMR reported by Turner and Hopkins (1974) are lower than the annual averages calculated for this study (Table 2). However, their station locations appear to have been near the mouth of the river which had the lowest average total abundance. Differences in counting methods must also be taken into account and perhaps more importantly, there is no information available on inter-annual variability for the LMR.

Seasonal phytoplankton community dynamics in the LMR and at the Tampa Bay station can be characterized as exhibiting a series of re-occurring blooms with a semi-monthly to monthly frequency. With the exception of the period of increased river flow in August-September and the Schizothrix bloom in Tampa Bay which invaded the river, there is no distinct seasonal pattern of abundance common to all river locations. However, populations of several species and groups displayed a seasonality in abundance and dominance that occurred at the Tampa Bay station and all river locations suggesting a linkage between the river and Tampa Bay.

The ubiquitous diatom, Skeletonema costatum, can be considered the dominant species during the entire year in the LMR and Tampa Bay. It was an important component (both numerically and as cell volume) of blooms at all stations. Steidinger and Gardiner (1985 and references therein) and Turner and Hopkins (1974) also described this species as the dominant in the entire Tampa Bay system. Blooms of other diatom species (e.g. Ceratulina bergonii, Thalassionema nitzschoides) contributed substantially to the community during summer blooms at salinity zones greater than 12<sup>0</sup>/oo. Several Chaetoceros spp. (C. subtilus, C. socialis and C. neogracile) were almost always present in all salinity zones and contributed to the dominance of diatom populations with respect to total cell volume (see Appendix 1). Microflagellates, which includes picoplankton sized cells (0.2 to 2.0um) were numerically dominant in Tampa Bay during spring and fall and in Ruskin Inlet during spring and summer. However, this group was the numerical dominant at all other LMR stations for most of the year. This was particularly true for the 12 and 6<sup>0</sup>/oo salinity zones where microflagellate populations contributed 76.1% and 65.3% of the total phytoplankton abundance, respectively. Diatoms contributed 31.4% of the total abundance at 0<sup>0</sup>/oo which contrasts with the intermediate salinity ranges where diatom abundance decreased (27.2% at 18<sup>0</sup>/oo, 17.5% at 12<sup>0</sup>/oo, and 21.3% at 6<sup>0</sup>/oo). Similar results for Tampa Bay were reported by Dragovich and Kelly (1964) and summarized by Steidinger and Gardiner (1985). In estuaries, coastal and oceanic waters this group can contribute 30 to 90% of the biomass and primary production (Malone, 1977, 1980; Durbin et al., 1975; Takahashi and Bienfang, 1983, Stockner, 1988). Although numerically important in the LMR and Tampa Bay, microflagellates did not dominate with respect to cell volume. Nutrient uptake rates and growth rates (to a limited extent, Banse, 1982) are cell size dependent and water column production has been related to cell volume and surface area (Smayda, 1965, Paasche, 1960). Measurements required to evaluate the role of microflagellates in the LMR phytoplankton community at the functional and process level have not been made, nor is there information available on their contribution to food web dynamics in the LMR system. Such information must be obtained for a complete evaluation of this group in the phytoplankton community dynamics of the Tampa Bay system.

The number of taxa recorded during this study suggests a high degree of species richness or diversity in the phytoplankton community. Calculations of diversity (Table 7), however, yield annual averages that range from 1.231 to 1.833. Margalef (1968) indicates values of 2.5 are common for actively growing coastal phytoplankton populations, 3.5 to 4.0 in the latter stages of succession and less than 2.5 for estuaries while ranges of 1 to 2.5 occur in eutrophic lakes. Ignatiades and Mimicos (1977) derived values of 3.15 and 2.37 for unpolluted and polluted coastal waters respectively. Similarly, indices in the range of 0.4 to 2.6 with an annual mean of 1.7 were calculated for the highly polluted Newark Bay, N.J. (McCormick and Quinn, 1975). Values for the LMR and Tampa Bay are within the same range as found in Newark Bay with annual averages of approximately 1.7. McCormick and Quinn (1975) used the same calculation of diversity as in this report and used Genus as the lowest taxonomic criterium. Therefore the Newark Bay and LMR diversity estimates are entirely comparable. Low diversity indices and high annual average biomass are indicative of an eutrophic estuary. Elevated nutrient levels, particularly phosphate, silicate and ammonium, are also indicative of an enriched estuary. The lack of nutrient limitation of short-term photosynthesis (discussed below) suggests nutrients were not limiting photosynthesis and were sufficient to support the phytoplankton standing crop.

Eutrophication within the LMR is, however, a matter of degree. Stations at 0<sup>0</sup>/oo and Ruskin Inlet, with elevated biomass, cell counts and production (0<sup>0</sup>/oo) are at one end of the spectrum, while the 18<sup>0</sup>/oo salinity zone was at the other. This latter station had the lowest total abundance, chlorophyll concentration, production and highest PI in the river (Table 2); characteristics which are not indicative of eutrophication. Annual mean values for ammonium and phosphate at Ruskin Inlet, 18<sup>0</sup>/oo and 12<sup>0</sup>/oo were 0.072 and 0.351, 0.088 and 0.333, 0.087 and 0.324 mg l<sup>-1</sup>, respectively. Silicate concentrations were higher at Ruskin Inlet and 12<sup>0</sup>/oo than at 18<sup>0</sup>/oo (3.54, 3.03 and 2.21 mg l<sup>-1</sup>, respectively), but this element was always available in excess. Since the average extinction coefficient at 18<sup>0</sup>/oo yields a 1% light level of 3.9 meters, well below the average bottom depth for this salinity zone, factors other than light or nutrients must be governing phytoplankton

abundance and production. Although the conclusion regarding the LMR as a eutrophic system may not apply directly to the 18<sup>0</sup>/oo salinity zone, excess nutrient availability and its diversity index does not exclude it.

Several other points regarding species composition in the LMR and Tampa Bay are of interest. Transport of the blue-green alga, Schizothrix, which invaded the river from Tampa Bay, the presence of Skeletonema costatum at the 0<sup>0</sup>/oo station and the annual average of 20% of Bay species at 0<sup>0</sup>/oo (Table 6) indicates significant interaction between the mouth and head of the river. Transport of freshwater species to Tampa Bay also occurred but, as noted above (see Similarity Index section), the presence of several species of estuarine diatoms, commonly found in Tampa Bay suggests upstream transport does occur. Although the S. costatum bloom in October at the fresh water station coincided with a bloom at all other stations and Tampa Bay, and there is a question about the viability of this species at this low salinity, its presence is relevant to any future studies or models of carbon flux and trophic interactions in the LMR.

Phytoplankton biomass, as indicated by chlorophyll levels, is primarily a product of growth in the river system. Chlorophyll concentrations at the Wimauma station (see Fig. 22), which is upstream from all LMR salinity zones, were considerably lower than the 0<sup>0</sup>/oo salinity zone and all other regions of the LMR. Therefore, elevated biomass in the LMR is the result of growth within the river system.

The eutrophic character of Ruskin Inlet is further emphasized by the almost year-round presence of two motile Chlorophytes, Eutreptia sp. and Euglena sp.. Both genera are phagotrophic and are associated with areas of high organic pollution (S. Wicks, pers. comm.). Attention should also be paid to the presence of Prorocentrum minimum as a member of the community in Ruskin Inlet. This species is responsible for massive red-tide blooms in other areas (e.g. Chesapeake Bay: Tyler and Seliger, 1981) and is euryhaline and eurythermal.

Another species of interest is the chain forming pennate diatom, Nitzschia pungens. This species was a significant component of the July 28 bloom in Tampa Bay and occurred at all other stations except 0<sup>0</sup>/oo. It is now known to produce domoic acid, a toxic excitatory amino acid,

which has recently been implicated as the causative agent of Amnesic Shellfish Poisoning. Over 100 people from Prince Edward Island, Canada became intoxicated with symptoms that, in their advanced stage, cause memory loss (Smith et al., 1989; Subba Rao and deFritas, 1989 and Todd, 1989). Little information is available about the quantity of toxin produced, clonal variation or the population levels necessary to cause shellfish toxicity.

#### Nutrient addition experiments

The use of short-term photosynthesis measurements with nutrient additions as a measure of potential nutrient limitation has been summarized by Elrifí and Turpin (1987) for nitrogen and by Lean and Pick (1981) for phosphorus in fresh waters. In both cases, photosynthesis is reduced over the short-term as a result of competition for available carbon skeletons and directing available energy to nutrient uptake when cells are nutrient limited. This reduction in carbon-14 uptake was measured in the first tens of minutes to 2-3 hours by Elrifí and Turpin (1987) after the addition of nitrate, nitrite or ammonium to nitrogen deficient cells and within the first 3 hours for phosphorus additions to cultures and natural populations of freshwater phytoplankton (Lean and Pick, 1981). Uptake rates of N or P by nitrogen and phosphorus limited cells in the light are enhanced relative to nutrient sufficient cells (see Morris, 1980 and Harris, 1986), which, over the long term (24 hours or longer), results in enhanced photosynthesis and biomass proportional to the concentration of the limiting nutrient.

Enhancement of carbon-14 uptake in the dark upon the addition of ammonium was used as an indicator of N-limitation in cultures and natural populations by Morris et al. (1971) and Yentsch et al. (1977). Elrifí and Turpin (1987) subsequently postulated a mechanism for this enhanced CO<sub>2</sub> uptake in the dark and demonstrated enhancement in the ratio of dark uptake with and without ammonium in cultures over a range of N-limited growth. The ratio increased to approximately 3 in populations under severe nitrogen limitation while a ratio of 1 was indicative of nutrient sufficient populations.

Enrichment of samples from Tampa Bay and 3 LMR stations with nitrate and phosphate does not show consistent reduction (or enhancement) of photosynthesis. There is a general trend toward a reduction of photosynthesis with both N and P addition at all stations, however this cannot be statistically demonstrated. When significant (with respect to a difference greater than 2) reduction or enhancement did occur, the results were not consistent with water column nutrient levels.

The enhancement of dark carbon-14 uptake upon the addition of ammonium did yield consistent ratios greater than 1 at all stations for a majority of sampling dates. With the exception of D+/D- ratios (CPM with ammonium/CPM without ammonium) greater than 2 at 18°/oo on July 28 and at 12°/oo on May 18 (Table 8), all ratios were less than 2. Using the results reported by Morris et al. (1971) and Elfifi and Turpin (1987) as a basis for determining N-limited populations, communities in Tampa Bay and the LMR could be considered nutrient sufficient or borderline N-limited for short-term photosynthesis. This conclusion is supported by the average annual PI, which for all locations was greater than 8 (Table 2). Values of 3 or less are generally indicative of nutrient limited populations or populations from oligotrophic waters, whereas values above 5 are generally indicative of nutrient replete populations (Curl and Small, 1965; Malone, 1971; Eppley et al., 1973). Additionally, when environmental conditions do not limit photosynthesis, there should be a direct relationship between the photosynthetic rate and biomass. This relationship was examined for Tampa Bay and the LMR production stations (Figs. 126 to 130). In Tampa Bay, the relationship yield a correlation coefficient ( $r^2$ ) of 0.64 if the two outlying points from May 4 and October 11 are deleted. At 18°/oo, 12°/oo and 0°/oo, the  $r^2$  values are 0.59, 0.96 and 0.83, respectively (Figs. 127, 128 and 130). All suggest, especially the relationships at 12°/oo and 0°/oo, that production was governed by the biomass present with no nutrient limitation. Light was constant during the incubation periods and there was no relationship between the PI and temperature (e.g. at 12°/oo, Fig. 129). If we assume that our experiments measured something close to the real  $P_{max}$  value for the water column then, the results suggest that neither temperature nor nutrients were limiting short-term

photosynthesis rates in Tampa Bay or the 3 LMR salinity zones.

This consistency in the PI index, especially for the 12<sup>0/00</sup> and 0<sup>0/00</sup> salinity zones, is even more remarkable given the changes in community composition based on numerical abundance that occurred at these and other stations. Variations in PI with both the size structure and composition of phytoplankton communities are well documented (see Malone, 1980a,b). Taguchi (1976) reported that  $P_{\max}$  normalized to chlorophyll (i.e. the PI index) decreased rapidly with cell size between 5 and 10  $\mu\text{m}$  spherical diameter and remained relatively constant for species with diameters between 25 and 170  $\mu\text{m}$ . Although microflagellates were the numerical dominants at 12<sup>0/00</sup> throughout the year, S. costatum or other diatoms dominated with respect to cell volume. This implies that the diatom populations were of primary importance from a physiological and, therefore, process standpoint. This remains to be tested. Another interpretation of this consistency in the PI is that all components of the community were equally efficient at photosynthesis under the prevailing environmental conditions.

#### POTENTIAL FOR EUTROPHICATION IN THE LMR

Although the data suggests that short-term, potential photosynthesis was not nutrient limited, this should not be taken to imply that limitation of longer-term growth or biomass did not occur. Discontinuous nutrient supplies, patchiness (both temporal and spatial) and high uptake rates during periods of nutrient availability will allow cells to fill internal storage compartments which can then be utilized to maintain photosynthetic rates (see Eppley, 1981). Coastal and oceanic phytoplankton populations can exhibit photosynthetic rates, uptake rates and compositional ratios which are indicative of nutrient sufficient populations (Sakshaug, 1980; Eppley, 1981) when in situ nutrient levels are virtually undetectable. As long as biomass remains low and relatively constant (at steady-state as in a chemostat), resupply rates via remineralization processes regulate growth and production (Smayda, 1983). Increasing the rate of remineralization or the absolute availability (concentration) will allow an increase in growth and, therefore, biomass. In this sense, the steady-state



population may not exhibit indications of nutrient limitation but growth and biomass would be considered as limited.

Examination of the water column concentrations of ammonium, nitrate, total dissolved inorganic nitrogen (DIN), and phosphate (Figs. 131 to 154), indicates that phosphate is always present in excess while ammonium, nitrate and DIN concentrations vary considerably and are at times at the limits of detection in the LMR and Tampa Bay. Ammonium concentrations, however, vary widely and contribute most of the nitrogen to the total DIN supply. Although there is usually measureable DIN in the water column, the ratio of Nitrogen:Phosphorus (N:P) either as nitrate or DIN were low with values less than 2 for most of the year in the LMR and Tampa Bay. Ratios of the same magnitude were compiled by Fanning and Bell (1985) and by Turner and Hopkins (1974) for Tampa Bay and its tributaries. They also describe their relationship with potential nitrogen limitation of phytoplankton communities. Under conditions of low N:P ratios, increasing the concentration of available nitrogen should yield increased biomass.

Nitrogen additions to samples collected at the 12<sup>0</sup>/oo salinity zone in the LMR and Tampa Bay are being conducted as part of the second year study. Preliminary results for the first five months are shown in Appendix 2. With the exception of the April sample in both Tampa Bay and the river, nitrogen additions yield a dramatic increase in chlorophyll levels, particularly in the greater than 12  $\mu$ m size fraction. This size fraction is dominated by chain-forming diatoms, particularly S. costatum and Chaetoceros spp.. Both can be considered r-selected species (Kilham and Kilham, 1980) that can outcompete others due to high nutrient unlimited growth rates. The addition of silicate alone and in combination with 25  $\mu$ M nitrogen does not increase yield over the nitrogen addition alone (Appendix 2). High silicate levels in the LMR and Tampa Bay (Figs. 155 to 160) with concomittent low N:S1 ratios should favor the selection of diatoms over other groups of phytoplankton (Sommer, 1986; Doering et al., 1989) which will dominate the community if their intrinsic growth rates are high. If silicate concentrations in the LMR were low, with concomittent low nitrogen levels, then groups such as flagellates or nitrogen fixing blue-green algae would have the ability to outcompete diatom populations.

Blue-greens and other nuisance algae predominate in lakes at N:P ratios higher than found in Tampa Bay and the LMR (Schindler, 1977; Barica et al., 1980). Therefore it is plausible to suggest that the community structure in the LMR and Tampa Bay, with a dominance by diatom populations and a "normal" community composition of flagellates and diatoms is dependent upon the availability of silicate.

Increased nitrogen loading of this estuarine system will increase the phytoplankton yield as our Year 2 experiments demonstrate and as the low N:P ratios suggest. The fact that this report suggests the phytoplankton community is nutrient sufficient with respect to short-term photosynthesis does not negate the previous statement. Little to no growth is observed in samples receiving no nitrogen addition in our current work. Therefore, the community is at steady-state with respect to in situ nutrient levels, or some other environmental factor is limiting growth. Since nitrogen addition alone increases yield, other growth factors are not limiting. The potential for increased eutrophication in the LMR and Tampa Bay is, therefore, high if increased nitrogen loading occurs.

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Table 1 : Location of Little Manatee River salinity stations and the Tampa Bay station by Riverrmile. Riverrmiles were measured as distance from the mouth. Negative numbers indicate distance into Tampa Bay measured in the channel from navigation marker #1. Positive numbers are distances from the mouth toward the head of the river.

Date/Station	T. Bay	18%	12%	6%	0%	R. Inlet
01/26/88	-2.27*	-0.22	N.D.	N.D.	5.7	2.50*
02/10		-0.31	N.D.	N.D.	6.1	
02/24		0.44	1.95	3.40	6.5	
03/09		0.00	0.83	3.00	4.65	
03/22		-1.82	-0.18	1.53	4.15	
04/06		0.00	2.55	6.00	7.2	
04/20		1.30	3.60	6.25	8.45	
05/04		1.85	3.70	6.15	8.35	
05/18		2.55	6.10	7.23	10.23	
06/01		3.70	5.65	8.30	10.46	
06/15		4.80	6.60	8.60	10.45	
06/29		5.10	6.16	9.60	10.63	
07/14		3.50	5.19	8.34	9.85	
07/28		1.40	3.50	5.30	7.19	
08/10		0.00	0.34	1.15	4.24	
08/30		-1.23	-0.91	0.00	3.40	
09/08		-2.10	T. Bay	-0.81	0.0	
09/22		T. Bay	1.53	4.10	6.25	
10/11		T. Bay	-0.45	4.20	6.25	
10/25		-0.19	1.00	2.72	7.10	
11/07		-1.45	-1.18	1.40	4.55	
11/21		-0.37	0.95	4.70	7.83	
12/08		-1.46	0.80	3.80	7.19	
12/20		0.00	1.51	4.65	7.19	
01/11/89		0.00	2.70	4.44	7.6	
01/24		T. Bay	-1.55	0.91	4.24	
Average	-2.27	+0.65	+2.19	+4.37	+6.76	+2.50
Range	0	-2.10to5.10	-1.55to6.60	-0.81to9.6	0.0to10.63	0

\*fixed location

Table 2 : Annual mean values for several parameters in the LMR and Tampa Bay arranged in rank order.

Total Counts		Chla		Extinction Coeff.		Photosynthesis		P.I.		
Rank	Station	cells ml <sup>-1</sup>	Station	µg l <sup>-1</sup>	Station	m <sup>-1</sup>	Station	mgCa <sup>-3</sup> hr <sup>-1</sup>	Station	mgCmgChl <sup>-1</sup> hr <sup>-1</sup>
1	R. Inlet	9391.1	0%	18.32	0%	-2.88	0%	122.25	18%	12.34
2	T. Bay	6007.0	R. Inlet	17.79	6%	-2.01	T. Bay	89.38	12%	11.41
3	0%	4712.1	6%	13.63	R. Inlet	-1.91	12%	84.85	T. Bay	10.66
4	6%	4314.0	12%	9.41	MOR	-1.61	18%	50.89	0%	8.79
5	12%	3260.8	T. Bay	7.46	12%	-1.54				
6	18%	2808.8	18%	4.72	18%	-1.19				
7					T. Bay	-1.15				



**Table 3 : Percent of surface irradiance at the maximum depth attained during the irradiance profile. Maximum depths were all within 0.5 m or less of the bottom.**

Date	T. Bay	MOR	18%	12%	6%	0%	R. Inlet
01/26/88	7.5	2.5	14.5	N.D.	N.D.	0.2	0.8
02/10	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
02/24	20.0	16.5	6.8	4.0	3.5	0.1	5.7
03/09	17.0	N.D.	10.0	2.9	0.3	0.05	0.8
03/22	24.6	5.3	9.6	5.0	4.3	0.6	0.6
04/06	8.3	N.D.	16.7	4.8	2.1	0.8	9.8
04/20	13.9	10.9	12.3	3.5	9.8	14.3	7.3
05/04	7.6	28.8	14.5	7.2	2.3	5.4	7.5
05/18	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
06/01	2.4	7.2	6.4	1.2	2.4	N.D.	2.7
06/15	0.6	9.9	11.5	0.5	3.9	8.5	1.5
06/29	0.6	12.7	2.2	7.9	1.4	17.5	5.2
07/14	1.5	6.5	3.1	1.1	1.2	4.9	4.9
07/28	5.2	8.2	4.8	1.3	0.3	0.02	2.7
08/10	4.1	N.D.	2.8	1.8	1.0	0.3	0.3
08/30	2.9	0.09	3.0	2.4	0.7	0.05	0.5
09/08	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
09/22	2.8	1.0	N.D.**	0.4	0.4	0.04	0.1
10/11	2.7	4.5	N.D.**	2.9	1.6	0.6	3.9
10/25	5.2	N.D.	4.9	1.8	9.3	0.08	0.9
11/07	5.1	1.7	1.9	5.8	1.6	4.5	1.2
11/21	7.7	10.4	9.6	2.3	4.1	0.6	2.3
12/08	14.3	5.7	9.6	0.4	4.7	3.4	4.4
12/20	11.2	N.D.*	16.2	11.4	1.5	8.3	4.2
01/11/89	6.5	N.D.*	10.1	1.9	10.4	30.2	6.7
01/24	9.8	0.7	N.D.**	7.4	7.8	1.3	0.5

N.D. = No Data

\*On this date the MOR station was equivalent to the 18% station.

\*\*On this date the 18% station was equivalent to Tampa Bay.

Table 4 : The annual mean cell concentration, the median cell concentration, and range, all as cells  $ml^{-1}$  for species that occurred  $\geq 13$  sample dates during 1988-1989 in the Little Manatee River. Data presented as: mean, median (range).

Group	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
flagellates	microflagellates	1664, 1906 (137-3777)	1581, 1585 (1-7011)	1925, 2185 (1075-4498)	2272, 3306 (1071-5783)	2713, 2485 (49-5326)	2881, 2699 (372-5919)
	unident. pennates	98, 14 (3-2003)				42, 36 (9-254)	
diatoms	Chaetoceros socialis	85, 70 (9-666)					
	Nitzschia closterium	89, 7 (1-950)	34, 3 (1-423)				
	Chaetoceros neogracile	41, 13 (1-754)					
	Leptocylindrus minimus	97, 17 (5-1978)					
	Ceratulina bergonii	44, 11 (1-943)					
	Skeletonema costatum	3309, 686 (40-23740)	429, 187 (52-3126)	296, 257 (71-1924)	365, 114 (0-2384)	1565, 349 (11-31331)	5147, 1310 (14-80358)
	Thalassionema nitzschooides	228, 33 (7-5419)					
	Chaetoceros subtilus	82, 28 (4-674)	165, 64 (5-1485)				
	Nitzschia longissima					4, 5 (1-43)	2, 4 (2-10)
	Rhizosolenia setigera	10, 11 (1-89)					
Dinoflagellates	Prorocentrum minimum	3, 3 (1-18)					95, 33 (2-1213)
	Prorocentrum micans	10, 8 (1-91)					
	Gymnodinium species					11, 7 (1-137)	
	Schroederia setigera				8, 6 (2-122)	12, 13 (1-117)	9, 5 (1-170)
Chlorophytes	Scenedesmus quadracauda				14, 3 (3-58)		
	Eutreptia species						38, 28 (5-403)

Table 5 : The frequency of occurrence for all species at each station. Values are the number of sampling dates the species was observed out of a total of 26 possible sample dates.

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
Diatoms							
102	unidentified pennates	14	9	10	7	18	9
105	Chaetoceros socialis	12	11	9	0	0	3
106	Nitzschia closterium	13	18	11	5	2	12
107	Chaetoceros neogracile	12	9	6	2	0	6
108	Nitzschia sigma	3	1	1	0	1	2
109	Leptocylindrus minimus	16	10	8	3	0	8
110	Leptocylindrus danicus	7	2	2	2	1	1
113	Ceratulina bergonii	18	7	6	2	0	5
114	Eucampia cornuta	3	1	0	0	0	0
115	Chaetoceros compressum	8	4	0	0	0	0
116	Rhizosolenia fragillissima	2	1	1	0	0	0
117	Thalassiosira oestrupii	5	3	1	0	0	1
118	Thalassiosira decipiens	10	2	1	7	6	11
119	Amphora species	6	3	1	0	0	3
120	Nitzschia longissima	12	8	11	12	13	13
121	Rhizosolenia calcar-avis	4	2	0	0	0	0
122	Rhizosolenia setigera	13	9	5	1	0	2
123	Skeletonema costatum	20	20	20	19	18	23
124	Rhizosolenia alata	4	1	0	0	0	0
125	Thalassiosira pseudonana	5	8	10	7	7	11
126	Rhizosolenia delicatula	5	1	0	0	0	1
127	Amphiprora species	4	2	1	2	11	1
128	Rhizosolenia hebetata	2	0	0	0	0	0
133	Amphiprora surirelloides	0	0	0	0	0	0
136	Rhizosolenia stouterforthii	2	1	0	0	0	0
137	Chaetoceros diversum	2	1	0	0	0	0
138	Amphora cymbelifera	1	0	0	0	0	1
139	Chaetoceros simplex	1	1	0	0	0	1
140	Thalassiosira rotula	1	1	0	0	0	1
142	Asterionella glacialis	10	3	0	0	1	0
143	Thalassiosira allenii	1	2	0	0	1	2
145	Dimerogramma fluvium	0	1	0	0	0	0
147	Melosira nummuloidea	2	1	0	0	3	1

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
150	Nitzschia pulchella	1	0	0	0	0	0
151	Chaetoceros danicum	2	1	0	0	0	0
152	Chaetoceros lorentzianus	4	5	0	0	0	0
153	Nitzschia pungens	11	4	5	3	0	2
154	Chaetoceros curvisetus	3	1	0	0	0	1
155	Chaetoceros subtilus	7	8	16	16	4	9
156	Chaetoceros polygonum	1	0	0	0	0	0
157	Dactylosolen mediterraneus	1	0	0	0	0	0
158	Chaetoceros didymum	2	0	0	0	0	0
160	Chaetoceros laciniosum	0	1	0	0	0	0
161	Amphiprora alata	0	1	0	0	0	0
163	Melosira varians	0	0	0	0	2	0
166	Nitzschia seriata	1	0	0	1	0	0
180	Coscinodiscus centralis	0	1	0	0	0	0
185	Synedra species	1	1	1	1	0	0
186	Coscinodiscus concinnus	0	1	0	0	0	0
190	Actinopterychus splendens	0	0	0	0	0	0
191	Thalassionema nitzschooides	14	6	4	1	0	3
194	Coretheron hystrix	2	0	0	0	0	0
196	Hemiaulis hauckii	3	0	0	0	0	0
197	Tropidoneis lepidoptera	1	0	0	1	2	0
203	Bacteriasstrum elongatum	1	0	0	0	0	0
204	Hemiaulis sinensis	1	0	0	0	0	0
208	Thalassiosira sp. (w/debris)	8	1	8	4	3	10
214	Guinardia flaccida	2	0	1	0	0	0
218	Coscinodiscus radiatus	0	0	1	0	0	0
222	Cyclotella meneghiniana	0	0	0	0	1	1
224	Nitzschia rigida	0	0	0	0	1	0
230	Biddulphia pulchella	1	0	0	0	0	0
231	Thalassiosira hyalina	1	0	0	0	0	0
236	Thalassiothrix frauenfeldianum	3	0	0	0	0	0
238	Bacillaria paxillifera	1	0	0	1	0	0
241	Skeletonema tropicum	0	0	0	0	0	0
242	Thalassiosira gravida	0	1	1	0	1	0
244	Coscinosira polychorda	3	0	0	0	0	0
248	Rhizosolenia inermis	0	0	0	0	1	0
249	Chaetoceros gracile	1	0	0	0	0	0

Diatoms

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
Diatoms							
250	Eucampia zoodiacus	1	0	0	0	0	0
251	Chaetoceros breve	1	1	0	0	0	0
253	Chaetoceros decipiens	0	0	1	0	0	0
254	Melosira species	4	0	0	2	3	0
257	Attheya species	0	0	0	0	1	0
263	Cyclotella species	0	3	4	5	12	6
267	Mastogloia species	0	1	0	0	0	0
273	Hantzschia species	0	0	0	0	0	1
278	Navicula species	3	1	2	0	0	0
279	Nitzschia species	1	0	2	0	1	0
280	Coscinodiscus species	3	0	0	0	0	1
281	Pleurosigma species	5	2	0	2	4	0
282	Chaetoceros species	5	2	2	0	0	0
284	Licmophora species	0	0	0	0	0	0
289	Gyrosigma species	1	0	0	1	1	1
295	Chaetoceros peruvianus	1	0	0	0	0	0
296	Thalassiosira aestivalis	0	0	0	0	0	1
298	Nitzschia lineata	0	0	0	0	1	0
299	Cyclotella species (small)	0	1	2	7	7	1
300	Thalassiosira species	1	0	0	0	0	0
301	Cyclotella species (large)	0	0	1	1	6	0
Microflagellates							
101	microflagellates	24	22	21	19	25	24
Dinoflagellates							
103	burst dinoflagellate	5	0	3	7	1	3
111	Prorocentrum minimum	13	6	6	4	3	15
112	Prorocentrum micans	15	4	4	0	0	3
134	Peridinium nipponicum	0	1	0	0	0	0
144	Peridinium turbo	1	0	0	0	0	0
146	Gonyaulax polyedra	0	1	0	0	0	0
148	Peridinium tuba	5	2	1	0	0	0
162	Peridinium trochoideum	0	0	0	0	1	2
164	Ceratium hircus	11	3	2	0	0	1
165	Oxytoxum scolopax	1	0	0	0	0	0
167	Proocentrum gracile	2	1	1	0	0	0
187	Gonyaulax grindlyi	0	0	1	0	0	0
188	Peridinium aciculiferum	1	8	10	8	1	11

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
Dinoflagellates	189 Gonyaulax diacantha	0	0	0	0	0	0
	195 Peridinium concinnum	3	0	1	2	0	1
	198 Gonyaulax spinifera	0	3	4	0	0	2
	201 Peridinium depressum	1	0	0	0	0	0
	202 Peridinium claudicans	1	1	0	0	0	0
	205 Peridinium divergens	2	0	0	0	0	0
	206 Peridinium oblongum	1	1	0	0	0	0
	207 Dinophysis caudata	2	1	1	0	0	2
	209 Amphidinium globosum	0	0	1	0	0	0
	211 Gonyaulax polygramma	3	4	3	7	0	3
	212 Gymnodinium estuariale	0	0	0	0	0	0
	215 Gonyaulax digitalis	2	0	1	1	0	2
	216 Peridinium brochii	0	0	1	0	0	0
	219 Gonyaulax monilata	3	1	4	0	0	1
	229 Oxytoxum gigas	1	0	0	0	0	0
	262 Amphidinium species	1	0	0	1	0	0
	276 Gymnodinium species	5	5	7	9	13	9
	283 Oxytoxum species	1	0	0	0	0	0
	287 Scrippsiella species	5	4	4	2	4	9
	291 Coolia species	2	1	0	0	0	0
Blue-greens	292 Peridinium species	7	4	3	5	0	6
	303 Peridinium crassipes	0	0	1	0	0	0
	104 Blue-green filaments	1	1	0	2	6	4
	171 Merismopedia punctata	1	0	0	2	6	5
	210 Anabaenopsis trichomes	0	0	0	0	0	0
	217 Schizothrix species	8	5	6	4	0	4
	221 Spirulina species (trichomes)	0	0	0	0	0	1
	227 Oscillatoria species (trichomes)	0	0	0	0	0	2
	228 Nostoc species (trichomes)	3	1	1	3	2	4
	258 Microcystis species	0	0	0	0	1	0
Chlorophytes	270 Ochromidium species (trichomes)	0	0	0	0	1	0
	100 Crucigenia species	1	0	0	1	7	5
	130 Akistrodesmus species	0	1	2	0	0	1
	131 Euglena species	0	2	2	4	5	6
	135 Scenedesmus quadracauda	1	1	2	9	14	5
	149 Asterococcus superbus	0	0	0	0	1	0

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Rushin Inlet
168	Scenedesmus acuminatus	0	1	1	3	6	2
169	Crucigenia fenestrata	0	0	1	0	0	0
170	Tetrastrum staurogeniaeforme	0	0	1	0	0	0
172	Akistrodesmus gracilis	0	0	0	1	3	1
173	Akistrodesmus falcatus	1	0	0	5	11	6
174	Clostridium species	0	0	0	0	1	0
175	Scenedesmus bijinga	1	1	0	4	11	4
176	Schroederia setigera	2	0	6	14	14	13
177	Phacus pleuronectus	0	0	1	0	1	0
178	Selenastrum gracile	0	0	0	1	4	2
179	Tetraedron species	0	0	0	0	1	1
181	Scenedesmus actus	0	1	1	1	2	0
182	Tetrastrum species	0	0	0	1	3	1
183	Akistrodesmus convolulus	0	0	0	0	0	1
184	Tetrastrum heterocontum	0	0	0	0	2	0
192	Schroederia anchora	0	0	0	0	1	0
193	Scenedesmus arcuatus	0	0	0	0	2	0
199	Crucigenia quadratus	0	0	0	1	0	1
213	Staurastrum paradoxym	1	0	0	0	0	1
220	Scenedesmus abundans	0	0	0	0	3	0
223	Agmenellum quadrupliatum	0	0	0	0	0	1
226	Franceia tuberculata	0	0	0	0	1	0
233	Akistrodesmus acuminatus	0	0	0	0	2	0
234	Actinastrum hantzschii	0	0	0	0	3	0
235	Microactinium pusillum	0	0	0	0	3	0
237	Pediastrum borfanum	0	0	0	0	1	0
239	colonial chlorophyte	0	0	0	1	1	0
240	Scenedesmus denticulus	0	0	0	0	1	0
243	Akistrodesmus fractus	0	0	0	0	1	1
246	Treubania crassispina	0	0	0	0	0	1
247	Polyedriopsis spinulosa	0	0	0	0	1	0
252	Pediastrum simplex	0	0	0	0	0	1
255	Pediastrum species	0	0	0	0	0	1
256	Gloenkia species	0	0	1	0	0	0
259	Oocystis species	0	0	0	0	0	1
261	Phacus species	0	1	0	0	4	1
264	Dictyosphaerium species	0	0	0	0	2	0

Chlorophytes

TAXCD	Species	Tampa Bay	18%	12%	6%	0%	Ruskin Inlet
Chlorophytes							
	265 Westella species	0	0	0	0	2	0
	268 Scenedesmus species	0	1	1	0	3	1
	269 Gloeocapsa species	0	0	0	0	1	0
	274 Selenastrum species	0	0	0	0	1	0
	275 Francella species	0	0	0	0	1	1
	277 Micratinium species	0	0	0	0	1	0
	285 Eutreptia species	2	5	9	5	11	15
	286 unknown Chlorophyte	0	0	0	0	2	0
	288 Actinastrum species	0	0	0	1	0	0
	290 Desmidiium species	0	0	0	0	0	0
	293 Kirchneriella species	0	0	0	0	1	0
	302 unidentified Chlorophyte	0	0	0	0	1	0
	304 Crucigenia tetrapedia	0	0	0	0	1	0
Silicoflagellates							
	132 Dictyocha fibula	2	0	1	0	0	0
	141 Ebria tripartita	3	2	2	0	0	0
Chrysophytes							
	266 Dinobryon species	0	0	0	0	2	1
	297 unknown Chrysophyte	0	0	0	0	0	1
Prymesioophyte							
	271 Pyramimonas species	1	1	0	1	1	3



**Table 6 : Similarity index of comparisons between community composition at Tampa Bay with all LMR stations.**

DATE	Station					
	MOR	18%	12%	6%	R. Inlet	0%
<b>1988</b>						
1/26	0.51	0.60			0.49	0.22
2/10	0.60	0.64			0.44	0.16
2/24	0.57	0.47			0.37	0.22
3/9		0.45	0.48		0.33	0.19
3/22	0.57	0.62	0.70		0.35	0.22
4/6		0.61	0.44		0.49	0.21
4/20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5/4		0.43	0.50	0.24	0.62	0.19
5/18		0.60	0.20	0.14	0.33	0.18
6/1		0.40	0.25	0.14	0.50	0.18
6/15		0.25	0.22	0.13	0.22	0.29
6/29		0.50	0.33	0.24	0.50	0.13
7/14		0.36	0.34	0.26	0.41	0.19
7/28		0.57	0.38	0.23	0.37	0.19
8/10		N.D.	0.55	0.34	0.20	0.09
8/30		0.62	0.53	0.21	0.18	0.26
9/8		0.44	N.D.	0.40	0.34	0.36
9/22		0.32	0.51	0.59	0.32	0.20
10/11	0.35	N.D.	0.52	0.37	0.27	0.16
10/25		0.36	0.33	0.08	0.31	0.24
11/7		0.50	0.36	0.23	0.22	0.12
11/21		0.24	0.29	0.16	0.23	0.24
12/8		0.48	0.27	0.14	0.47	0.27
12/20		0.42	0.11	0.25	0.36	0.17
<b>1989</b>						
1/11		0.23	0.31	0.25	0.39	0.24
1/24	0.24	N.D.	0.53	0.31	0.32	0.21
Annual Mean		0.445	0.388	0.248	0.360	0.205

N.D. = No Data

**Table 7** : Values for the Shannon-Weaver diversity index ( $\bar{H}$ ) for the combined replicate counts for each LMR station. Identification to Genus was the lowest taxonomic criterium used for the calculation; therefore "unidentified" groups and microflagellates were not included.

DATE	TAMPA BAY	MOR	18%	$\bar{H}$ 12%	9%	6%	R. Inlet	0%
<b>1988</b>								
1/26	-0.450	-2.279	-0.839		-1.673		-2.226	-2.863
2/10	-0.684	-1.211	-1.312				-0.895	-2.137
2/24	-0.577	-1.020	-1.268		-2.079		-1.309	-0.938
3/9	-2.385		-2.445	-2.986			-1.936	-0.973
3/22	-3.029	-2.559	-4.068	-3.174			-2.415	-2.448
4/6	-1.452		-1.794	-2.016			-1.641	-1.899
4/20	--	--	--	--	--	--	--	--
5/4	-1.214		-0.670	-2.285		-1.701	-1.195	-1.534
5/18	-0.360		-1.454	-1.279		-2.104	-0.768	-0.264
6/1	-1.023		-1.830	-0.903		-1.259	-0.141	-1.421
6/15	-0.000		-1.049	-0.484		-1.413	-0.082	-2.157
6/29	-0.492		-1.272	-1.556		-1.916	-0.120	-1.803
7/14	-2.927		-0.457	-1.747		-1.764	-0.619	-2.232
7/28	-0.858		-1.877	-2.399		-2.045	-1.525	-2.090
8/10	-0.909		N.S.	-1.609		-2.607	-4.599	-2.566
8/30	-1.879		-3.010	-2.313		-2.193	-2.535	-1.777
9/8	-1.844		-1.499	N.D.		-2.142	-2.102	-1.621
9/22	-1.249			-1.786		-0.726	-1.653	-2.812
10/11	-1.526	-0.689	N.D.	-0.549		-0.878	-0.044	-0.610
10/25	-2.052		-2.495	-1.520		-1.491	-1.209	-1.291
11/7	-0.845		-1.586	-1.226		-1.924	-0.390	-2.596
11/21	-1.288		-1.128	-1.403		-1.662	-1.725	-1.899
12/8	-2.032		-2.055	-1.374		-1.338	-1.941	-1.679
12/20	-0.341		-1.944	-1.887		-2.013	-2.177	-2.089
<b>1989</b>								
1/11	-0.963		-2.829	-1.478		-2.127	-2.177	-2.089
1/24	-2.440	-1.992	N.D.	-2.100		-2.038	-1.348	-2.643
Annual Mean	-1.231		-1.757	-1.718		-1.755	-1.409	-1.833

N.D. = No Data  
N.S. = Not Sampled

**Table 8** : Ratios of dark carbon -14 uptake with and without the addition of ammonium. Ratios are expressed as: CPM with ammonium / CPM without ammonium (D+/D-) and as the average uptake rate in the dark bottles with ammonium minus the average uptake rate in dark bottles without ammonium ( $V_{D+} - V_{D-}$ ;  $\text{mgCm}^{-3}\text{hr}^{-1}$ ).

	T. Bay		MOR		9%		0%	
DATE	D+/D-	$V_{D+} - V_{D-}$	D+/D-	$V_{D+} - V_{D-}$	D+/D-	$V_{D+} - V_{D-}$	D+/D-	$V_{D+} - V_{D-}$
1988								
1/26	1.18	+0.32	1.09	+0.02	0.92	-0-	-0-	-0-
			18%					
1988								
2/10	1.58	1.17	1.98	+1.25	0.73	0.90	0.48	N.S.
					12%			
1988								
2/24	1.21	0.14	0.76	-0-	1.15	0.11	1.57	0.54
3/9	1.39	0.46	1.11	0.13	1.28	0.78	1.34	0.34
3/22	1.29	0.46	1.68	0.92	1.49	0.45	1.59	0.52
4/6	1.19	0.47	1.24	0.93	1.81	1.68	1.59	1.42
4/20	1.14	0.37	1.32	0.42	1.30	0.52	1.39	0.86
5/4	1.73	0.85	1.62	0.87	1.17	0.30	1.38	0.74
5/18	0.85	-0-	1.34	0.69	2.34	1.91	1.72	1.58
6/1	1.47	1.32	1.32	0.70	1.23	0.39	0.99	-0-
6/15	1.09	0.37	1.61	1.37	1.14	0.49	0.91	-0-
6/29	1.36	0.86	1.66	1.46	0.98	-0-	0.97	-0-
7/14	0.73	-0-	1.63	1.20	1.91	3.81	1.16	0.30
7/28	1.35	1.03	2.21	2.89	0.82	-0-	1.48	0.39
8/10	1.14	0.68	0.69	-0-	1.38	1.44	1.06	0.11
8/30	1.12	1.16	0.93	-0-	1.04	0.22	0.90	-0-
9/18	1.38	0.28	0.92	-0-	N.S.	N.S.	1.19	0.09
9/22	0.82	-0-	N.S.	N.S.	0.82	-0-	1.13	0.44
10/11	1.69	2.35	N.S.	N.S.	0.86	-0-	1.12	0.26
10/25	1.27	0.92	1.06	0.17	1.08	0.24	1.49	1.36
11/7	1.56	0.13	1.43	0.38	1.27	0.30	1.32	0.34
11/21	1.13	0.14	1.19	0.19	1.13	0.18	1.86	1.10
12/8	1.41	0.35	1.11	0.10	1.38	0.30	1.63	0.83
12/20	1.05	0.06	1.37	0.32	0.95	-0-	1.26	9.24
1989								
1/11	1.54	0.59	1.28	0.28	1.62	0.76	1.24	0.44
1/24	0.63	-0-	N.S.	N.S.	0.78	-0-	1.02	0.02

-0- = L-D = 0 or negative

N.S. = Not Sampled

